Short Communication

Response of *Chironomus striatapennis* larvae exposed to three heavy metals

Author details: Rahul Podder 1, Susanta Nath 2*, Biplob Kumar Modak 3and Sudipta Das 4

1, 3 Department of Zoology, Sidho-Kanho-Birsha University, Purulia, 723 104, West Bengal, India

2 Department of Zoology, Government G.D. College Singur, 712 409, West Bengal, India

4 Department of Zoology, Durgapur Government College, 713 214, West Bengal, India

* Email: susanta_nath@yahoo.com

ABSTRACT

In this experiment, estimation of LC_{50} of Lead (Pb), Cadmium (Cd) and Mercury (Hg) was carried out when *Chironomus striatapennis* was exposed to different treatment doses. Chi square was used to test for heterogeneity and the result was found to be significant ($p<0.05$) in all three metals. Fourth instar larvae were collected from breeding aquarium under laboratory conditions and exposed for 96 hours to different doses of Pb, Cd and Hg for static bioassay to measure the LC_{50}. Ten fourth instar larvae were placed in 100 ml beaker with 50 ml of each test solution. Larvae were exposed to six different concentrations, consisting of five trials. A control was also maintained wherein organisms were exposed to distilled water. Larvae were not fed during the toxicity tests. All beakers were free from tube forming materials. Data of mortality were subjected to probit analysis. Results showed that sensitivity of larvae to metals was Hg> Cd >Pb. *C. striatapennis* showed noticeable response in LC_{50} study and was sensitive to low doses of heavy metals. Several secondary consumers have preferred this larva as their food. So unplanned industrialization may increase the level of heavy metals in the aquatic ecosystem which will accumulate slowly but definitely in

different trophic level and at the same time unusual death of these larvae may indirectly change the equilibrium of the aquatic ecosystem.

Key Words: LC_{50}, Mercury, Lead, Cadmium, *Chironomus striatapennis.*

INTRODUCTION

Fresh and marine waters are polluted daily by untreated or improperly treated industrial wastewater. Over 80% of the world's wastewater and over 95% in some least developed countries is released wastewater to the environment without treatment [1]. It is estimated that in India 13,500 million litres of industrial wastewater is generated per day in urban cities and discharged into nearby aquatic bodies with or without treatment [2]. Industrial wastes from different industries, such as mining operations, metal plating, radiator manufacturing, tanneries, smelting and alloy industries, storage battery industries are the significant sources of heavy metals [3]. Among the heavy metals, Cd, Pb and Hg, are considered as most hazardous water pollutants [4, 5]. Due to their high solubility in water, heavy metals could be absorbed by living organisms once they enter the aquatic food chain [6]. Benthic primary consumer like chironomid larvae (Order Diptera, Family Chironomidae) are continuously exposed to such environments, and may contribute to the accumulation and bio transfer of these heavy metals to upper trophic level. They are thus considered as good biological indicator of aquatic environment degradation [7, 8, 9]. *Chironomus striatapennis* was found highly sensitive when exposed to different doses of Arsenic salt [10]. LC_{50} is a statistical parameter which illustrates a complete picture of mortality in a population and also organism's tolerance to a particular xenobiotic [11]. The objective of the study is to determine the LC_{50} when *C. striatapennis* is exposed to concentrations of Pb, Cd and Hg. The work is also aimed at finding how this macroorganism is responding to these heavy metals. This in turn would provide information regarding the level of these metals in the industrial effluents which would not be deleterious to this primary consumer.

MATERIAL AND METHODS

Collection of Chironomid larvae

Fourth instar larvae of *Chironomous striatapennis* were collected from fresh water pond located at Kanchrapara (22_56018.664800N, 88_28010.034400E) district of North 24 Parganas, West Bengal, India and placed in aerated plastic bags and transported to the laboratory. Larvae of chironomid were reared under laboratory conditions by using the breeding aquarium which was filled to a depth of approximately 20 cm with pond water and given fish flakes for food [12].This was the source of all test organisms. Atomic absorption spectrophotometry was done to confirm that larvae were not contaminated with Lead (Pb), Mercury (Hg) and Cadmium (Cd).

Toxicity Test of Heavy metals

For contamination, stock of 1mg l^{-1} concentration was prepared initially with Cadmium acetate (SRL, 99% purity), Lead acetate (SRL, 99% purity) and Mercuric chloride (SRL, 98% purity) in double distilled water and kept for twenty four hours. Test solutions of different concentrations were prepared from that stock through a series of dilution. Initially a series of tests were conducted in concentrations ranged between 0.0005 mgl^{-1} and 1 mg l^{-1}, to which test organisms were exposed for 96 hours. Finally for Cd, concentrations of 0.001mg l^{-1}(d1), 0.003mg l^{-1}(d2), 0.007mg l^{-1}(d3), 0.015 mg l^{-1}(d4), 0.03mg l^{-1}(d5) and 0.062 mg l^{-1}(d6); for Hg,0.0005mg l^{-1}(d1), 0.001mgl^{-1}(d2), 0.003mg l^{-1}(d3), 0.007 mgl^{-1}(d4), 0.015mg l^{-1}(d5) and 0.031 mgl^{-1}(d6), and for Pb,0.003mgl^{-1}(d1) 0.007mg l^{-1}(d2), 0.015mg l^{-1}(d3), 0.031 mgl^{-1}(d4), 0.062mg l^{-1}(d5) and 0.125 mg l^{-1}(d6) were considered for the experiment. Ten, fourth instar larvae were placed in 100 ml beaker with 50 ml of each test solution. Each concentration consists of five trials. A control was also maintained wherein organisms were exposed to distilled water. Larvae were not fed during the toxicity test. All beakers were free from tube forming materials. The criterion for death is immobility and/or lack of reaction to mechanical stimulus. After 96 hours, recorded data were subjected to probit analyses [13] by using Probit Programme Version 1.5.

RESULTS

LC_{50} and LC_{90} values and 95% confidence limit for Hg, Cd and Pb in the fourth instar of *Chironomus striatapennis* are presented in Table 1. The result revealed that sensitivity of larvae was Hg>Cd>Pb. Chi-square for Heterogeneity were also found significant in all three metals in comparison to tabulated value of Chi-square (7.815, $P<0.05$). Percentage of mortality of larvae exposed to three heavy metals is presented in Fig.1.

Table 1: LC_{50}, LC_{90} and Confidence Limit of *C. striatapennis*

	Mercury	**Cadmium**	**Lead**
Exposer Period	96 Hour	96 Hour	96 Hour
LC_{50} mg l^{-1}	0.001	0.003	0.007
LC_{90} mg l^{-1}	0.010	0.012	0.104
95% Confidence Limit for LC_{50}	**Lower Limit:** 0.000	**Lower Limit:** 0.000	**Lower Limit:** 0.000
	Upper Limit: 0.003	**Upper Limit:** 0.005	**Upper Limit:** 0.019

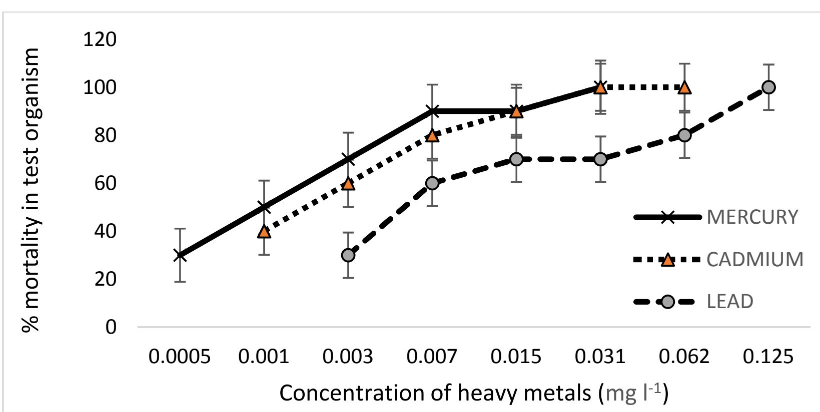

Fig. 1: Concentration of Hg, Cd,Pb and percentage mortality in *C striatapennis*

DISCUSSION

Mercury, a prevalent toxicant is present in the environment due to anthropogenic activity as well as from natural sources. Present study revealed that *C. striatapennis* was more susceptible to Hg than other two heavy metals and the observed LC_{50} exposed to Hg was 0.001 mgl^{-1}, which was same as human permissible limit (0.001mgl^{-1}) according to BIS [14] and less than acceptable limit in industrial effluent (0.01mgl^{-1}) [15]. In industrial effluent this metal may be available in higher concentration [16] and has been found to reduce growth and locomotion activity which lead to increase of the probability of mortality rate of the *Chironomous* larvae [17]. Moreover, increase in Hg concentration decreases the survival rate of this larva as was observed in *Eriocheir sinensis* [18].

The study revealed that *C. striatapennis* was more susceptible to Cd than Pb and LC_{50} of these three heavy metals showed that lead is least toxic for this insect. Toxic effect of cadmium reduced the uptake of essential metals, specifically Calcium (Ca) ion channel due to their similarity of size and charge which can disrupt the normal physiological actions of Ca ion. Cellular tolerance to Cd was probably due to high affinity sequestration of the toxic metal by Metallothionein (MT), a metal binding protein (MBP) which is present in *Chironomus* [19]. In spite of that, severe amount of cadmium may increase the mortality rate of this organism. Pb was found to be less toxic but it also had similar effect that prevented or imitated the action of Ca ion of Calcium-dependent or allied process [20]. Moreover, Pb accumulation by this larvae is higher than other heavy metals due to the presence of MBP which may cause bio- magnification of this heavy metal in the food chain [21].

Our study revealed that the lethal concentration of these metals (LC_{50}) obtained in this experiment, though below the human permissible limit was not suitable for the survival of the larvae of this insect. The maximum acceptable limit for Pb and Cd in industrial effluents are 0.1 mgl^{-1} and 0.01 mgl^{-1} respectively [22]. LC_{50} was found to be less than the acceptable limit. Whereas, LC_{90} for this insect was recorded for Pb (0.10 mgl^{-1}) and Cd (0.012 mgl^{-1}) which were similar to the acceptable limit in the industrial effluents.

Chironomids are macro benthos and are the primary consumer of the aquatic food chain [23]. In stress situation like increasing concentration of heavy metals in aquatic environment the level of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase in *Chironomus* are decreased and larvae died due to toxicity of the metals [24]. Due to unscrupulous industrialization in the developing countries, there is an increase in the industrial effluents containing heavy metals like Hg, Cd and Pb which ultimately pollute the fresh water sources of those areas. Bioaccumulation of these metals in aquatic organisms is dangerous not only for their own survival and biology, but also for humans because of the possible passage of contaminant through the food chain [25].

Though there is no noticeable changes found in higher vertebrates like fish in those aquatic ecosystems, but our LC_{50} results indicated that *C. striatapennis* was highly sensitive to low doses of heavy metals. Several secondary consumers consider chironomids as their food. So heavy metal pollution may indirectly distort the equilibrium of the aquatic ecosystem. This study provides information for industries to release effluents after proper treatment so that level of these heavy metals would remain below the effective level. That is essential for sustainable development and to stop the loss of biodiversity of the ecosystem.

CONCLUSION

LC_{50} assay revealed that larvae of *Chironomus striatapennis* was more sensitive to Hg than Cd and Pb respectively. It was also observed that LC_{50} values were less than standard permissible limit of these heavy metals. As this larvae is preferred by different secondary consumers, so unplanned industrialization may increase the level of heavy metals in the aquatic ecosystem which will accumulate slowly but definitely in different trophic levels and at the same time unusual death of these larvae may indirectly change the equilibrium of the aquatic ecosystem.

Ethical Disclaimer:

As per international standard or university standard, written ethical approval has been collected and preserved by the author(s).

Consent Disclaimer:

NA

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